FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **11002/002001**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/355220**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP98/00268	**20 January 1998**	**24 January 1997**

TITLE OF INVENTION

NOVEL METHOD FOR THE ISOLATION OF POLYSACCHARIDES

APPLICANT(S) FOR DO/EO/US

THOMAS HASLER; EMIL FURER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
    A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☒ Certificate of Mailing by Express Mail
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Date of Deposit 23 JULY 1999

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Mike Angelastro

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U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/355220	PCT/EP98/00268	11002/002001

20. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

	CALCULATIONS PTO USE ONLY
☐ Search Report has been prepared by the EPO or JPO **$930.00**	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) **$720.00**	
☐ No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) **$790.00**	
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO **$1,070.00**	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) **$98.00**	

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CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	14 - 20 =	0	x $22.00	**$0.00**
Independent claims	1 - 3 =	0	x $82.00	**$0.00**
Multiple Dependent Claims **(check if applicable)**.		☐		**$0.00**

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Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐ **$0.00**

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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

John R. Wetherell, Jr., Ph.D.
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4225 Executive Square, Suite 1400
La Jolla, California 92037

SIGNATURE

John R. Wetherell, Jr., Ph.D.
NAME

31,678
REGISTRATION NUMBER

7/22/99
DATE

PATENT
ATTORNEY DOCKET NO. 11002/002001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Thomas Hasler et al. Art Unit: Not Known
Serial No.: Not yet assigned Examiner: Not Known
Filed : 23 July 1999
Title : NOVEL METHOD FOR THE ISOLATION OF POLYSACCHARIDES

Box PCT

Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Prior to examination and calculation of fees for the above-identified application, please enter the following amendments and remarks:

In the claims:

Please cancel claims 1-22, without prejudice.

Please add claims 23-36 as follows:

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Date of Deposit 23 JULY 1999
I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as "**Express Mail Post Office To Addressee**" with sufficient postage on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Mike Augustine
M. E. Augustine

--23. A method for the isolation of polysaccharides, wherein the following steps are carried out:

(a) mixing of a bacterial polysaccharide fraction with a detergent solution;

(b) addition of alcohol to a final concentration which is below the concentration at which the polysaccharide precipitates;

(c) mixing the solution;

(d) filtering the solution by way of a deep bed filter;

(e) separation of the polysaccharide from detergent and alcohol.

24. The method of claim 23, wherein the alcohol is ethanol.

25. The method of claim 23, wherein the separation of the polysaccharide is carried out by the precipitation of the polysaccharide by adding more alcohol.

26. The method of claim 23, wherein the polysaccharides stem from gram-negative bacteria.

27. The method of claim 26, wherein the gram-negative bacteria are selected from the genus consisting of Haemophilus, Neisseria, Klebsiella and Escherichia.

28. The method of claim 23, wherein the detergent is an anionic surfactant.

29. The method of claim 28, wherein the anionic surfactant is an alkyl sulfate, for example sodium dodecyl sulfate (SDS).

30. The method of claim 28, wherein the surfactant concentration in the solution added to the polysaccharide fraction in step (a) is at the most 20% (w/w/).

31. The method of claim 30, wherein the surfactant concentration in the polysaccharide solution is 0.1% to 4% (final concentration, w/w).

32. The method of claim 23, wherein in step (b) the alcohol is added to the solution to a final concentration which is approximately 10 % below the concentration at which the polysaccharide precipitates.

33. The method of claim 23, wherein the initial concentration of polysaccharides in the polysaccharide fraction is greater than 10 mg/ml.

34. The method of claim 23, wherein the filtration is carried out by means of a polymer filter.

35. The method of claim 23, wherein the polymer filter and/or the deep bed filter is a polypropylene filter.

36. The method of claim 26, wherein the gram-negative bacteria is selected from the group consisting of Haemophilus influenzae (type b), Klebsiella pneumoniae, Neisseria meningitidis and Escherichia coli.--

If the Examiner would like to discuss any of the issues raised in this Amendment, Applicant's representative can be reached at (619) 678-5070.

Please charge any additional fees, or make any credits, to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 7/23/99

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PC/EP98/00268
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Our Ref.: C 1047 PCT

Translation (sg)

Novel Method for the Isolation of Polysaccharides

The invention relates to a method for the isolation of polysaccharides, in particular for the separation of endotoxins from capsule polysaccharides of gram-negative bacteria. The polysaccharides isolated by this method are preferably used for the production of polysaccharide based vaccines. The invention furthermore relates to vaccines containing polysaccharides isolated by the method described in this invention.

In producing vaccines, in particular polysaccharide vaccines from bacterial polysaccharides, the removal of endotoxins is a critical and decisive step during the purification of polysaccharides. The method for the separation of endotoxins from bacterial polysaccharides which is used most often according to the state of the art is based on the extraction with phenol, which, if necessary, has to be repeated several times until the endotoxin content is as required by health authorities. This method is complicated and time-consuming. In addition, working with phenol is troublesome and causes undesirable toxic waste. Moreover, the yields of polysaccharides obtainable by said methods known from the state of the art are often unsatisfactory. Other methods for

the isolation of bacterial polysaccharides known from the state of the art are based on the use of affinity columns. These are often injurious to health (e.g. the use of column material containing polymyxin B). Furthermore, many column materials have only a limited capacity, which, for obtaining technically usable yields of polysaccharides, necessitates large and thus expensive columns (cf. e.g. US-A 5,045,456; US-A 5,039,610; and US-A 5,034,519).

Therefore, the problem to be solved by the invention was to provide a method for the isolation of polysaccharides which is simple, economically useful and less injurious to health. The solution to said problem is achieved by the embodiments characterized in the claims.

Thus, the invention relates to a method for the isolation of polysaccharides, wherein the following steps are carried out:

(a) mixing of a bacterial polysaccharide fraction with a detergent solution;
(b) addition of alcohol to a final concentration which is below the concentration at which the polysaccharide precipitates;
(c) mixing the solution;
(d) filtering the solution;
(e) separation of the polysaccharide from detergent and alcohol.

Bacterial polysaccharide fractions which can be used in the method of the invention can be produced by the methods known from the state of the art; cf. e.g. Gotschlich et al., J. Exp. Med. 129 (1969), 1349-1365 as well as Schneerson et al., J. Exp. Med. 152 (1980), 361-376. The concentration of alcohol at which the polysaccharide precipitates in the presence of a detergent solution can be determined by the person skilled in the art according to conventional methods. For example, this concentration can be determined by way of simple series of tests.

The reaction, i.e. the precipitation of the endotoxin from the polysaccharide solution, is conventionally carried out from 1 minute up to 1 hour; it can, however, be carried out for

several hours. In contrast to the methods known from the state of the art, the method of the invention is simple, fast, inexpensive and causes less toxic waste. In addition, the yields of polysaccharide are considerably higher. The method of the invention is based on a selective precipitation of alcohol in the presence of at least one detergent which abolishes non-covalent interactions between polysaccharides, lipopolysaccharides and proteins.

In a preferred embodiment of the method of the invention the alcohol to be added is ethanol.

In a further preferred embodiment of the method of the invention the separation of the polysaccharide from detergent and alcohol is carried out by the precipitation of the polysaccharide by adding more alcohol.
This embodiment of the method is particularly preferred as the precipitation of the polysaccharide and thus the separation of detergents and alcohol can be achieved by simply adding more alcohol. In another embodiment, precipitation of the polysaccharide can be achieved by adding alcohol different from the one used in step (b).

A further preferred embodiment of the invention relates to a method, wherein the polysaccharides stem from gram-negative bacteria. In a particularly preferred embodiment the gram-negative bacteria are bacteria of the genus Haemophilus, Neisseria, Klebsiella or Escherichia and in particular of the species Haemophilus influenzae (type b), Neisseria meningitidis, Klebsiella pneumoniae or Escherichia coli. The polysaccharides here concerned are capsule polysaccharides.

The isolation of polysaccharides from bacteria of these genera and/or species is particularly preferred as these polysaccharides are suitable for use in the vaccination against the following diseases: meningitis, epiglottitis, otitis media, pneumonia, arthritis, sepsis, nosocomial infections, urinary tract infections and gastroenteritis.

In a further preferred embodiment of the method of the invention the detergent is an anionic surfactant. Particularly preferred is a method wherein the anionic surfactant is an alkyl sulfate, for example sodium dodecyl sulfate (SDS).
The advantage of the use of SDS in the method of the invention is i.a. that SDS is obtainable from a plurality of manufacturers at a favorable price.

In a further particularly preferred embodiment of the method of the invention the surfactant concentration in the solution added to the polysaccharide fraction in step (a) above is at the most 20 % (w/w). As mentioned above, the surfactant is preferably an alkyl sulfate and for example SDS.

Particularly preferred as the method of the invention is a method wherein the surfactant concentration in the polysaccharide solution, for example the SDS concentration, is 0.1% to 4 % (final concentration, w/w).

In an additional preferred embodiment of the method of the invention the alcohol is added in step (b) to the solution to a final concentration which is approximately 10 % below the concentration at which the polysaccharide precipitates.
It has been found by way of empirical series of tests that the addition of alcohol in step (b) to this final concentration is particularly advantageous as the loss of polysaccharide in the presence of this concentration is small, and endotoxin nonetheless is efficiently precipitated.

In another preferred embodiment of the method of the invention the initial concentration of polysaccharides in the polysaccharide fraction is greater than 10 mg/ml.
While the method of the invention can as well be carried out at smaller concentrations of polysaccharides in the polysaccharide fraction, the above-mentioned concentration should be used as minimum concentration in the method of the invention, in particular for economic reasons.

In a further preferred embodiment, the method of the invention relates to a method wherein the filtration is carried out by means of a polymer filter.

In another preferred embodiment of the method of the invention, filtration is carried out by means of a deep bed filter.

In the context of the present invention, the term "deep bed filter" means a filter which in contrast to a membrane filter (2-dimensional) possesses a 3-dimensional structure (depth). This structure has the advantage that the deep bed filter has a high capacity of retaining particles and correspondingly does not become obstructed so fast.

In the present invention, the use of polymer or deep bed filters has proved its worth. In this context, it has to be noted that a polymer filter can as well be a deep bed filter, and vice-versa, a deep bed filter can be a polymer filter; this condition, however, is not obligatory.

The isolation of the polysaccharides according to the method of the present invention is particularly efficient when deep bed filters are used for filtration.

In a particularly preferred embodiment of the method of the invention the polymer filter and/or the deep bed filter is a polypropylene filter.

The invention further relates to a polysaccharide vaccine which comprises a polysaccharide isolated according to the method of the invention. Optionally, said polysaccharide vaccine also includes a pharmaceutically acceptable carrier. Examples of such carriers are tetanus toxoid, diphteria toxoid, Pseudomonas Exotoxin A and cholera toxin.

The polysaccharide vaccine of the invention, as can be taken from the above explanations, can be produced in a particular simple and inexpensive manner. In addition, its production is particularly unharmful for the laboratory staff's health. An example of the vaccine of the present invention is a vaccine based on meningococci polysaccharide. These as well as the below-mentioned embodiments of the vaccine of the invention are administered parenterally, the administration being effected one or several times and preferably (where not indicated differently) several times. Usually, the administration is effected intramuscularly or subcutaneously, wherein per dosis 1-50 µg polysaccharide are used.

The polysaccharide vaccine of the invention is particularly suitable for the vaccination against meningitis, epiglottitis, otitis media, pneumonia, arthritis, sepsis, nosocomial infections, urinary tract infections or gastroenteritis. Moreover, the vaccine of the invention can also be used for the vaccination against other diseases caused by gram-negative bacteria carrying capsule polysaccharides.

Furthermore, the invention relates to a conjugate consisting of a polysaccharide isolated according to the method of the invention and a pharmaceutically acceptable protein chemically connected therewith. Examples of such proteins are tetanus toxoid, diphteria toxoid, Pseudomonas Exotoxin A and cholera toxin. Preferred dosages comprise 1-20µg of the conjugate.

Additionally, the invention relates to a conjugate vaccine comprising a polysaccharide isolated according to the method of the invention and a pharmaceutically acceptable protein chemically connected therewith.

The conjugate vaccine of the invention is preferably used for the immunization against or prophylaxis of the diseases mentioned above.

In this context, it is particularly preferred that the immunization is carried out with small children.

The invention further relates to a combination vaccine comprising a polysaccharide isolated according to the method of the invention or a conjugate of the invention as well as an additional immunogenic component, wherein the additional immunogenic component preferably induces an immune response against a pathogen different from the pathogen from which the polysaccharide stems. An example of a combination vaccine is a Haemophilus influenzae vaccine in which the corresponding polysaccharide is conjugated with tetanus toxoid. E.g. pertussis, diphteria, tetanus and hepatitis B components may additionally be formulated in said vaccine. Preferred dosages comprise 1-20 µg of polysaccharide in the combination vaccine, particularly preferred are 1-10 µg, for example in the case of the Haemophilus influenzae combination vaccine

described above, of Haemophilis influenzae polysaccharide. Preferred dosages for diphteria components in said vaccine are 15-25 Lf (Limit of flocculation), for tetanus components 5-10 Lf and for pertussis components more than 4 IU (International Units). The person skilled in the art can determine the dosages/concentrations of additional components in the combination vaccine of the invention according to standard procedures/standard provisions. The combination vaccine of the invention is preferably administered only once.

The additional immunogenic component is preferably a diphteria, tetanus, pertussis, hepatitis B or poliomyelitis antigen.

Finally, the invention relates to the use of a polysaccharide isolated according to the method of the invention as intermediate product for the production of a conjugate or combination vaccine. Here, the intermediate product is chemically connected with a pharmaceutically acceptable protein so as to form a conjugate. Correspondingly, the invention preferably relates to a use, wherein the conjugate or combination vaccine comprises as an active component a conjugate comprising a polysaccharide isolated according to the method of the present invention and a pharmaceutically acceptable protein chemically connected therewith.

The examples illustrate the invention.

Example 1

Isolation of a Haemophilus influenzae type b capsule polysaccharide

A capsule polysaccharide fraction (PRP, polyribosylribitolphosphate) from Haemophilus influenzae type b processed according to conventional methods is mixed in a concentration of > 10 mg/ml with a 4% SDS solution. Ethanol is then added to a final concentration which is about 10% below the concentration at which the polysaccharide begins to precipitate. The solution is mixed for about 20 minutes, time periods of from 1 minute to several hours also appearing suitable, followed by a slight turbidity.

Thereupon, filtration is effected by way of a polypropylene deep bed filter. By this filtration step, the endotoxins are separated and remain in the filter. It is to be expected that filtration as well as adsorption effects are responsible for the separation of the endotoxins. The filtered polysaccharide then precipitates by further adding ethanol, the SDS remaining in the solution. The precipitated polysaccharide can be separated from remaining SDS impurities by further ethanol precipitations. An additional processing of the polysaccharide as well as the fabrication as a vaccine, wherein the polysaccharide is preferably chemically connected with a suitable carrier protein, is carried out according to conventional methods known from the state of the art. In the preferred embodiment mentioned, the polysaccharide is also an intermediate product for a conjugate vaccine.

Example 2

Isolation of Neisseria meningitidis type (A) and (C) capsule polysaccharides

Neisseria meningitidis type (A) and (C) capsule polysaccharides were subjected to the same method steps as described in Example 1.

The yields of polysaccharides obtained by the methods described in Examples 1 and 2 are depicted in Tables I and II. It can be observed that the yield of Haemophilus influenzae type (b) capsule polysaccharides obtainable by the method of the invention is considerably higher than polysaccharide obtainable by methods known from the state of the art (phenol extraction).

Table I

Isolation of H. influenzae type b capsule polysaccharide (PRP)

PRP Lot number[1]	Method	Amount of PRP (g)	Endotoxin before (EU/µg/PRP)	Endotoxin after (EU/µg/PRP)	Yield of PRP (%)
27	5 x phenol	8.3	475	26	67
627095	EtOH/SDS	1.9	72.5	0.11	>95
611496	EtOH/SDS	75	55	<0.05	>95

[1] The lot numbers are numbers used internally by the applicant, CH-Serum. The lots were prepared according to conventional methods.

Table II

Isolation of N. meningitidis group C capsule polysaccharide (GCMP)

GCMP Lot number[1]	Method	Amount of GCMP (g)	Endotoxin before (EU/µg/GCMP)	Endotoxin after (EU/µg/GCMP)	Yield of GCMP (%)
150396	EtOH/SDS	7.3	46.8	7.7	92
905096	EtOH/SDS	7.5	258	1.1	77
906096	EtOH/SDS	7.8	85	0.1	67

Claims

1. A method for the isolation of polysaccharides, wherein the following steps are carried out:
   (a) mixing of a bacterial polysaccharide fraction with a detergent solution;
   (b) addition of alcohol to a final concentration which is below the concentration at which the polysaccharide precipitates;
   (c) mixing the solution;
   (d) filtering the solution;
   (e) separation of the polysaccharide from detergent and alcohol.

2. The method of claim 1, wherein the alcohol is ethanol.

3. The method of claim 1 or 2, wherein the separation of the polysaccharide is carried out by the precipitation of the polysaccharide by adding more alcohol.

4. The method of any one of claims 1 to 3, wherein the polysaccharides stem from gram-negative bacteria.

5. The method of claim 4, wherein the gram-negative bacteria are bacteria of the genus Haemophilus, Neisseria, Klebsiella or Escherichia and in particular of the species Haemophilus influenzae (type b), Klebsiella pneumoniae, Neisseria meningitidis or Escherichia coli.

6. The method of any one of claims 1 to 5, wherein the detergent is an anionic surfactant.

7. The method of claim 6, wherein the anionic surfactant is an alkyl sulfate, for example sodium dodecyl sulfate (SDS).

8. The method of claim 6 or 7, wherein the surfactant concentration in the solution added to the polysaccharide fraction in step (a) is at the most 20% (w/w).

9. The method of claim 8, wherein the surfactant concentration in the polysaccharide solution is 0.1 % to 4 % (final concentration, w/w).

10. The method of any one of claims 1 to 9, wherein in step (b) the alcohol is added to the solution to a final concentration which is approximately 10 % below the concentration at which the polysaccharide precipitates.

11. The method of any one of claims 1 to 10, wherein the initial concentration of polysaccharides in the polysaccharide fraction is greater than 10 mg/ml.

12. The method of any one of claims 1 to 11, wherein the filtration is carried out by means of a polymer filter.

13. The method of any one of claims 1 to 12, wherein the filtration is carried out by means of a deep bed filter.

14. The method of claim 12 or 13, wherein the polymer filter and/or the deep bed filter is a polypropylene filter.

15. A polysaccharide vaccine comprising a polysaccharide isolated according to the method of any one of claims 1 to 14 and, optionally, a pharmaceutically acceptable carrier.

16. The polysaccharide vaccine of claim 15 for the prophylaxis against meningitis, epiglottitis, otitis media, pneumonia, arthritis, sepsis, nosocomial infections, urinary tract infections or gastroenteritis.

17. A conjugate comprising a polysaccharide isolated according to the method of any one of claims 1 to 14 and a pharmaceutically acceptable protein chemically connected therewith.

18. A conjugate vaccine comprising a polysaccharide isolated according to the method of any one of claims 1 to 14 and a pharmaceutically acceptable protein chemically connected therewith.

19. A combination vaccine comprising a polysaccharide isolated according to the method of any one of claims 1 to 14 or a conjugate of claim 17 as well as at least one further immunogenic component.

20. The combination vaccine of claim 19, wherein the additional immunogenic component is a diphteria, tetanus, pertussis, hepatitis B or poliomyelitis antigen.

21. Use of a polysaccharide isolated according to the method of any one of claims 1 to 14 as intermediate product for the production of a conjugate or combination vaccine.

22. The use of claim 20, wherein the conjugate or combination vaccine comprises as an active component a conjugate comprising a polysaccharide isolated according to the method of any one of claims 1 to 14 and a pharmaceutically acceptable protein chemically connected therewith.

Abstract

Novel Method for the Isolation of Polysaccharides

The invention relates to a method for the isolation of polysaccharides, in particular for the separation of endotoxins from capsule polysaccharides of gram-negative bacteria. The polysaccharides isolated by this method are preferably used for the production of polysaccharide vaccines. The invention furthermore relates to vaccines containing polysaccharides isolated by the method described in this invention.

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled NOVEL METHOD FOR THE ISOLATION OF POLYSACCHARIDES, the specification of which

☐ is attached hereto.

■ was filed on July 23, 1999 as Application Serial No. 09/355,220
and was amended on _____.

■ was described and claimed in PCT International Application No. PCT/EP98/00268 filed on 1/20/98 and was amended on 1/18/99.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

COUNTRY	APPLICATION NO.	FILING DATE	PRIORITY CLAIMED
EPO	97101143.2	1/24/97	■ Yes ☐ No

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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